

SEQUENCE LISTING

<110> Skeiky, Yasiko Guderian, Jeffremank Off Corixa Corporation

<120> Methods of Using a Mycobacterium tuberculosis Coding Sequence to Facilitate Stable and High Yield Expression of Heterologous Proteins

<130> 014058-008010US

<140> US 09/684,215

<141> 2000-10-06

<150> US 60/158,585

<151> 1999-10-07

<160> 22

<170> PatentIn Ver. 2.1

<210> 1

<211> 1872

<212> DNA

<213> Mycobacterium tuberculosis

<220>

<223> 32 KD serine protease MTB32A

<220>

<221> CDS

<222> (89)..(1156)

<223> MTB32A

<220>

<221> sig_peptide

<222> (89)..(184)

<223> N-terminal hydrophobic secretory signal sequence

<220>

<221> mat_peptide

<222> (185)..(1153)

<400> 1

gactacgttg gtgtagaaaa atcctgccgc ccggaccctt aaggctggga caatttctga 60

tagctacccc gacacaggag gttacggg atg agc aat tcg cgc cgc cgc tca 112

Met Ser Asn Ser Arg Arg Arg Ser

-30 -25

ctc agg tgg tca tgg ttg ctg agc gtg ctg gcc gtc ggg ctg ggc $\,$ 1 Leu Arg Trp Ser Trp Leu Leu Ser Val Leu Ala Ala Val Gly Leu Gly

-20 -15 -10

RECEIVED

MAR 1 9 2002

TECH CENTER 1600/2900

							ctg Leu									256
							gtc Val									304
							acc Thr									352
_		_					gtg Val				_			_		400
							caa Gln 80									448
							gcg Ala									496
ggc Gly 105	ctg Leu	ccg Pro	tcg Ser	gcg Ala	gcg Ala 110	atc Ile	ggt Gly	ggc Gly	ggc Gly	gtc Val 115	gcg Ala	gtt Val	ggt Gly	gag Glu	ccc Pro 120	544
_	_		_			_	ggt Gly		_			_				592
							ctc Leu									640
_	_			_	_		aca Thr 160									688
							tcg Ser									736
gga Gly 185	cag Gln	gtg Val	gtc Val	ggt Gly	atg Met 190	aac Asn	acg Thr	gcc Ala	gcg Ala	tcc Ser 195	gat Asp	aac Asn	ttc Phe	cag Gln	ctg Leu 200	784
							gcc Ala									832
							ggt Gly									880
							ttg Leu 240									928

	*				
ggc gca cga g Gly Ala Arg y 250	gtc caa cgc gt Val Gln Arg Va 25	l Val Gly Se	c gct ccg gcg g r Ala Pro Ala A 260	ca agt ctc la Ser Leu	976
			g gtc gac ggc g a Val Asp Gly A 275		1024
aac tog goo a Asn Ser Ala	acc gcg atg gc Thr Ala Met Al 285	g gac gcg ct a Asp Ala Le 29	t aac ggg cat c u Asn Gly His H O	at ccc ggt is Pro Gly 295	1072
Asp Val Ile S			g tog ggc ggc ac s Ser Gly Gly Tl 33		1120
	aca ttg gcc ga Thr Leu Ala Gl		g gcc tga tttcg o Ala	cgcg	1166
gataccaccc go	ccggccggc caat	tggatt ggcgc	cagee gtgattgee	g cgtgagcccc	1226
cgagttccgt c	tcccgtgcg cgtg	gcatcg tggaa	gcaat gaacgaggca	a gaacacagcg	1286
togagcacco to	cccgtgcag ggca	gtcacg tcgaa	ggcgg tgtggtcgag	g catccggatg	1346
ccaaggactt c	ggcagcgcc gccg	ccetge cegec	gatec gacetggtt	aagcacgccg	1406
tettetaega ge	gtgctggtc cggg	cgttct tcgac	gccag cgcggacggt	teeggegate	1466
tgcgtggact ca	atcgatcgc ctcg	actacc tgcag	tggct tggcatcgad	tgcatctggt	1526
tgccgccgtt ct	tacgactcg ccgc	tgcgcg acggc	ggtta cgacattcgo	gacttctaca	1586
aggtgctgcc cg	gaattegge aceg	tcgacg atttc	gtcgc cctggtcgad	gccgctcacc	1646
ggcgaggtat co	cgcatcatc accg	acctgg tgatga	aatca caccteggaq	g tegeacecet	1706
ggtttcagga gt	tecegeege gace	cagacg gaccg	tacgg tgactattad	gtgtggagcg	1766
acaccagcga go	cgctacacc gacg	cccgga tcatc	ttegt egacacegaa	a gagtegaact	1826
ggtcattcga to	cctgtccgc cgac	agttct actgg	caccg attett		1872

<210> 2

<211> 355

<212> PRT

<213> Mycobacterium tuberculosis

<223> 32 KD serine protease MTB32A

<400> 2

 Met
 Ser
 Asn
 Ser
 Arg
 Arg
 Ser
 Leu
 Arg
 Trp
 Ser
 Trp
 Leu
 Leu
 Leu
 Ser

 Val
 Leu
 5
 10
 15
 15

 Val
 Leu
 Ala
 Val
 Gly
 Leu
 Gly
 Leu
 Ala
 Thr
 Ala
 Gln
 Ala
 Ala</

Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly Thr 7.0 75 Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His Val 90 85 Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly Gln 100 105 Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val Ala 120 Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile Gly 140 135 Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser Gly 150 155 Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala Leu 170 Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu Thr 190 185 180 Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp Ser 200 205 195 Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn Thr 220 210 215 Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gln Gly Phe Ala 230 235 Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg Ser Gly 250 245 Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly Leu 265 260 Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val Val 280 285 Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val Ile 300 295 Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala Asp 310 315 Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Thr Trp Gln 330 325 Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu Gly 345 Pro Pro Ala 355 <210> 3 <211> 396 <212> DNA <213> Mycobacterium tuberculosis <220> <223> 14 KD C-terminal fragment of MTB32A Ra12 <220> <221> CDS <222> (1)..(396) <223> Ra12 <400> 3 acg gcc gcg tcc gat aac ttc cag ctg tcc cag ggt ggg cag gga ttc Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gln Gly Phe gec att eeg ate ggg eag geg atg geg ate geg gge eag ate ega teg

(14)

Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg Ser

25

20

ggt ggg ggg tca ccc acc gtt cat atc ggg cct acc gcc ttc ctc ggc Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly ttg ggt gtt gtc gac aac aac ggc aac ggc gca cga gtc caa cgc gtg Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val gtc ggg agc gct ccg gcg gca agt ctc ggc atc tcc acc ggc gac gtg Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val 70 atc acc gcg gtc gac ggc gct ccg atc aac tcg gcc acc gcg atg gcg 288 Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala gac gcg ctt aac ggg cat cat ccc ggt gac gtc atc tcg gtg acc tgg 336 Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Thr Trp 100 105 caa acc aag tog ggc ggc acg cgt aca ggg aac gtg aca ttg gcc gag Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu 115 396 gga ccc ccg gcc Gly Pro Pro Ala 130 <210> 4 <211> 132 <212> PRT <213> Mycobacterium tuberculosis <223> 14 KD C-terminal fragment of MTB32A Ra12 Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg Ser 20 25 Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly 40 Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val 55 Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val 75 Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala 90 Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Thr Trp 105 Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu Gly Pro Pro Ala 130 <210> 5 <211> 702

<212> DNA

<213> Artificial Sequence

```
ccc ctc ccc ggc ccg cca ccg ggt ggt tgc ggt ggg gca att ccg
Pro Leu Pro Gly Pro Pro Pro Gly Gly Cys Gly Gly Ala Ile Pro
       210
                            215
                                                                  702
tcc gag cag ccc aac gct ccc tga gaattc
Ser Glu Gln Pro Asn Ala Pro
    225
                        230
<210> 6
<211> 230
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:Ra12-DPPD fusion
     polypeptide
<400> 6
Met His His His His His Thr Ala Ala Ser Asp Asn Phe Gln Leu
Ser Gln Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala
Ile Ala Gly Gln Ile Arg Ser Gly Gly Ser Pro Thr Val His Ile
                            40
Gly Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn
                        55
    50
Gly Ala Arg Val Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu
                                                            8.0
                    70
                                        75
Gly Ile Ser Thr Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile
                                                        95
                85
                                    90
Asn Ser Ala Thr Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly
                                                    110
                               105
           100
Asp Val Ile Ser Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr
                                                125
                           120
       115
Gly Asn Val Thr Leu Ala Glu Gly Pro Pro Ala Glu Phe Asp Asp Asp
                        135
                                           140
Asp Lys Asp Pro Pro Asp Pro His Gln Pro Asp Met Thr Lys Gly Tyr
                                       155
                    150
Cys Pro Gly Gly Arg Trp Gly Phe Gly Asp Leu Ala Val Cys Asp Gly
                165
                                   170
Glu Lys Tyr Pro Asp Gly Ser Phe Trp His Gln Trp Met Gln Thr Trp
                               185
                                                   190
Phe Thr Gly Pro Gln Phe Tyr Phe Asp Cys Val Ser Gly Gly Glu Pro
                           200
                                               205
Leu Pro Gly Pro Pro Pro Pro Gly Gly Cys Gly Gly Ala Ile Pro Ser
                        215
  210
Glu Gln Pro Asn Ala Pro
                    230
<210> 7
<211> 1746
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence:Ra12-WT1 fusion
<220>
<221> CDS
<222> (4)..(1740)
<223> Ra12-WT1 fusion polypeptide
```

												gga Gly		768
												tca Ser 270		816
												tgc Cys		864
	_	_	_		-	_			_		-	acc Thr	_	912
-		_	-									gcg Ala		960
												cag Gln		1008
												ggc Gly 350		1056
			_	_	_		-	_	_	_	-	ctg Leu		1104
												ctt Leu		1152
												ggc Gly		1200
												tgc Cys		1248
												cag Gln 430		1296
	_	_										gca Ala		1344
		_		_		_	_	-				tgc Cys		1392
_	_		_	-			_	_		_		aag Lys		1440

Thr Gly Glu Lys	cca tac Pro Tyr 485	cag tgt Gln Cys	gac tto Asp Phe	aag g Lys A 490	ac tgt sp Cys	gaa o Glu A	ga Arg	agg Arg 495	1488
ttt ttt cgt tca Phe Phe Arg Ser						His T			1536
gtg aaa cca ttc Val Lys Pro Phe 515	cag tgt Gln Cys	aaa act Lys Thr	tgt cag Cys Glr 520	cga a Arg L	ag ttc ys Phe	tcc c Ser A 525	egg Arg	tcc Ser	1584
gac cac ctg aag Asp His Leu Lys 530	acc cac Thr His	acc agg Thr Arg 535	act cat Thr His	aca g	gt gaa ly Glu 540	aag c Lys F	ro	ttc Phe	1632
agc tgt cgg tgg Ser Cys Arg Trp 545				Phe A					1680
tta gtc cgc cat Leu Val Arg His 560	cac aac His Asn 565	atg cat Met His	cag aga Gln Arg	aac a Asn M 570	tg acc let Thr	aaa c Lys L	etc Leu	cag Gln 575	1728
ctg gcg ctt tga Leu Ala Leu	gaattc								1746
<210> 8 <211> 578									
<212> PRT <213> Artificia <223> Descripti polypepti	on of Art		Sequenc	e:Ra12	-WT1 fu	sion			
<212> PRT <213> Artificia <223> Descripti	on of Art de	ificial					3ln 15	Leu	
<212> PRT <213> Artificia <223> Descripti polypepti <400> 8 Met His His His	on of Art de His His 5	ificial His Thr	Ala Ala	Ser A	sp Asn	Phe G	15		
<212> PRT <213> Artificia <223> Descripti polypepti <400> 8 Met His His His 1 Ser Gln Gly Gly	on of Art de His His 5 Gln Gly	ificial His Thr	Ala Ala 10 Ile Pro 25	Ser A	sp Asn	Phe G	15 let	Ala	
<212> PRT <213> Artificia <223> Descripti polypepti <400> 8 Met His His His 1 Ser Gln Gly Gly 20 Ile Ala Gly Gln	on of Art de His His 5 Gln Gly Ile Arg	ificial His Thr Phe Ala Ser Gly 40	Ala Ala 10 Ile Pro 25 Gly Gly	Ser A Ile G Ser P Val A	sp Asn ly Gln ro Thr 45	Phe G Ala M 30 Val H	15 Met Mis	Ala Ile	
<pre><212> PRT <213> Artificia <223> Descripti polypepti <400> 8 Met His His His 1 Ser Gln Gly Gly</pre>	on of Art de His His 5 Gln Gly Ile Arg Phe Leu Gln Arg 70	His Thr Phe Ala Ser Gly 40 Gly Leu 55 Val Val	Ala Ala 10 Ile Pro 25 Gly Gly Gly Val	Ser A Ile G Ser P Val A Ala P 75	sp Asn ly Gln ro Thr 45 sp Asn 60 ro Ala	Phe G Ala M 30 Val H Asn G	15 Met Mis Mis	Ala Ile Asn Leu 80	
<pre><212> PRT <213> Artificia <223> Descripti polypepti <400> 8 Met His His His 1 Ser Gln Gly Gly</pre>	on of Art de His His 5 Gln Gly Ile Arg Phe Leu Gln Arg 70	His Thr Phe Ala Ser Gly 40 Gly Leu 55 Val Val	Ala Ala 10 Ile Pro 25 Gly Gly Gly Val	Ser A Ile G Ser P Val A Ala P 75 Val A	sp Asn ly Gln ro Thr 45 sp Asn 60 ro Ala	Phe G Ala M 30 Val H Asn G	15 Met Mis Mis	Ala Ile Asn Leu 80	
<pre><212> PRT <213> Artificia <223> Descripti polypepti <400> 8 Met His His His 1 Ser Gln Gly Gly</pre>	on of Art de His His 5 Gln Gly Ile Arg Phe Leu Gln Arg 70 Gly Asp 85	His Thr Phe Ala Ser Gly 40 Gly Leu 55 Val Val Val Ile	Ala Ala 10 11e Pro 25 Gly Gly Gly Val Gly Ser Thr Ala 90	Ser A Ile G Ser P Val A Ala P 75 Val A	sp Asn ly Gln ro Thr 45 sp Asn 60 ro Ala sp Gly	Phe G Ala M 30 Val H Asn G Ala S Ala F	15 Met His His His His Horo 95	Ala Ile Asn Leu 80 Ile	
<pre><212> PRT <213> Artificia <223> Descripti polypepti <400> 8 Met His His His 1 Ser Gln Gly Gly</pre>	on of Art de His His 5 Gln Gly Ile Arg Phe Leu Gln Arg 70 Gly Asp 85 Ala Met	His Thr Phe Ala Ser Gly 40 Gly Leu 55 Val Val Val Ile Ala Asp	Ala Ala 10 11e Pro 25 Gly Gly Gly Val Gly Ser Thr Ala 90 Ala Leu 105	Ser A Ile G Ser P Val A Ala P 75 Val A Asn G	sp Asn ly Gln ro Thr 45 sp Asn 60 ro Ala sp Gly	Phe G Ala M 30 Val H Asn G Ala S Ala F His F	15 Met Mis Sly Ser Pro 95	Ala Ile Asn Leu 80 Ile Gly	
<pre><212> PRT <213> Artificia <223> Descripti polypepti <400> 8 Met His His His 1 Ser Gln Gly Gly</pre>	on of Art de His His 5 Gln Gly Ile Arg Phe Leu Gln Arg 70 Gly Asp 85 Ala Met Val Thr	His Thr Phe Ala Ser Gly 40 Gly Leu 55 Val Val Val Ile Ala Asp Trp Gln 120	Ala Ala 10 11e Pro 25 Gly Gly Gly Val Gly Ser Thr Ala 90 Ala Leu 105 Thr Lys	Ser A Ile G Ser P Val A Ala P 75 Val A Asn G Ser G Ala G	sp Asn Thr 45 sp Asn 60 ro Ala sp Gly His Hy Gly 125	Phe G Ala M 30 Val H Asn G Ala S Ala F His F 110 Thr A	15 Met Mis Sly Ser Pro 95 Pro	Ala Ile Asn Leu 80 Ile Gly Thr	
<pre><212> PRT <213> Artificia <223> Descripti polypepti <400> 8 Met His His His His 1 Ser Gln Gly Gly</pre>	on of Art de His His 5 Gln Gly Ile Arg Phe Leu Gln Arg 70 Gly Asp 85 Ala Met Val Thr Leu Ala Pro Met	His Thr Phe Ala Ser Gly 40 Gly Leu 55 Val Val Val Ile Ala Asp Trp Gln 120 Glu Gly 135	Ala Ala 10 11e Pro 25 Gly Gly Gly Val Gly Ser Thr Ala 90 Ala Leu 105 Thr Lys	Ser A Ile G Ser P Val A Ala P 75 Val A Asn G Ser G Ala G	sp Asn ly Gln ro Thr 45 sp Asn 60 ro Ala sp Gly ly His ly Gly 125 lu Phe 40	Phe G Ala M 30 Val H Asn G Ala S Ala F His F 110 Thr A Pro L	15 Met Mis Mis More Pro 95 Pro Arg Leu	Ala Ile Asn Leu 80 Ile Gly Thr	
<pre><212> PRT <213> Artificia <223> Descripti</pre>	on of Art de His His 5 Gln Gly Ile Arg Phe Leu Gln Arg 70 Gly Asp 85 Ala Met Val Thr Leu Ala Pro Met 150	His Thr Phe Ala Ser Gly 40 Gly Leu 55 Val Val Val Ile Ala Asp Trp Gln 120 Glu Gly 135 Gly Ser	Ala Ala 10 11e Pro 25 Gly Gly Gly Val Gly Ser Thr Ala 90 Ala Leu 105 Thr Lys Pro Pro Asp Val	Ser A Ile G Ser P Val A Ala P 75 Val A Asn G Ser G Ala G 11 Arg A 155 Gly G	sp Asn ly Gln ro Thr 45 sp Asn 60 ro Ala sp Gly ly His ly Gly 125 lu Phe 40 sp Leu	Phe G Ala M 30 Val H Asn G Ala S Ala F His F 110 Thr A Pro I Asn A	15 Met His	Ala Ile Asn Leu 80 Ile Gly Thr Val Leu 160	

```
Gly Ala Ser Ala Tyr Gly Ser Leu Gly Gly Pro Ala Pro Pro Pro Ala
               200
Pro Pro Pro Pro Pro Pro Pro Pro His Ser Phe Ile Lys Gln Glu
                     215
                                        220
Pro Ser Trp Gly Gly Ala Glu Pro His Glu Glu Gln Cys Leu Ser Ala
               230
                                    235
Phe Thr Val His Phe Ser Gly Gln Phe Thr Gly Thr Ala Gly Ala Cys
                               250
              245
Arg Tyr Gly Pro Phe Gly Pro Pro Pro Pro Ser Gln Ala Ser Ser Gly
                             265
          260
Gln Ala Arg Met Phe Pro Asn Ala Pro Tyr Leu Pro Ser Cys Leu Glu
                          280
Ser Gln Pro Ala Ile Arg Asn Gln Gly Tyr Ser Thr Val Thr Phe Asp
                                         300
                      295
Gly Thr Pro Ser Tyr Gly His Thr Pro Ser His His Ala Ala Gln Phe
                 310
                                    315
Pro Asn His Ser Phe Lys His Glu Asp Pro Met Gly Gln Gln Gly Ser
              325
                               330
Leu Gly Glu Gln Gln Tyr Ser Val Pro Pro Pro Val Tyr Gly Cys His
                             345
                                               350
Thr Pro Thr Asp Ser Cys Thr Gly Ser Gln Ala Leu Leu Leu Arg Thr
                         360
                                         365
Pro Tyr Ser Ser Asp Asn Leu Tyr Gln Met Thr Ser Gln Leu Glu Cys
                     375
                                         380
Met Thr Trp Asn Gln Met Asn Leu Gly Ala Thr Leu Lys Gly His Ser
                                     395
                  390
Thr Gly Tyr Glu Ser Asp Asn His Thr Thr Pro Ile Leu Cys Gly Ala
              405
                                 410
Gln Tyr Arg Ile His Thr His Gly Val Phe Arg Gly Ile Gln Asp Val
                                                430
                             425
          420
Arg Arg Val Pro Gly Val Ala Pro Thr Leu Val Arg Ser Ala Ser Glu
                         440
Thr Ser Glu Lys Arg Pro Phe Met Cys Ala Tyr Ser Gly Cys Asn Lys
                     455
                                        460
Arg Tyr Phe Lys Leu Ser His Leu Gln Met His Ser Arg Lys His Thr
         470 475
Gly Glu Lys Pro Tyr Gln Cys Asp Phe Lys Asp Cys Glu Arg Arg Phe
             485
                                 490
Phe Arg Ser Asp Gln Leu Lys Arg His Gln Arg Arg His Thr Gly Val
                              505
                                                510
Lys Pro Phe Gln Cys Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp
                                             525
                         520
His Leu Lys Thr His Thr Arg Thr His Thr Gly Glu Lys Pro Phe Ser
                      535
                                        540
Cys Arg Trp Pro Ser Cys Gln Lys Lys Phe Ala Arg Ser Asp Glu Leu
                                    555
                550
Val Arq His His Asn Met His Gln Arg Asn Met Thr Lys Leu Gln Leu
                                 570
             565
Ala Leu
<210> 9
<211> 672
```

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Ral2-human mammaglobin
fusion

```
<210> 10
<211> 220
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:Ral2-human mammaglobin
     fusion polypeptide
<400> 10
Met His His His His His Thr Ala Ala Ser Asp Asn Phe Gln Leu
                                    10
Ser Gln Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala
Ile Ala Gly Gln Ile Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile
                            4.0
Gly Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn
                        55
Gly Ala Arg Val Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu
                                        75
Gly Ile Ser Thr Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile
                85
                                    90
Asn Ser Ala Thr Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly
                               105
                                                   110
           100
Asp Val Ile Ser Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr
       115
                           120
                                               125
Gly Asn Val Thr Leu Ala Glu Gly Pro Pro Ala Glu Phe Ile Glu Gly
                       135
                                           140
Arg Gly Ser Gly Cys Pro Leu Leu Glu Asn Val Ile Ser Lys Thr Ile
                                      155
                  150
Asn Pro Gln Val Ser Lys Thr Glu Tyr Lys Glu Leu Leu Gln Glu Phe
                                   170
               165
Ile Asp Asp Asn Ala Thr Thr Asn Ala Ile Asp Glu Leu Lys Glu Cys
                              185
                                                  190
Phe Leu Asn Gln Thr Asp Glu Thr Leu Ser Asn Val Glu Val Phe Met
                        200
      195
Gln Leu Ile Tyr Asp Ser Ser Leu Cys Asp Leu Phe
                       215
    210
<210> 11
<211> 2191
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence:Ra12-H9-32A fusion
     (Ra12-MTB39-MTB32A(N-ter) fusion)
<220>
<221> CDS
<222> (1)..(2190)
<223> Ra12-H9-32A (Ra12-MTB39-MTB32A(N-ter)) fusion polypeptide
atg cat cac cat cac cat cac acg gcc gcg tcc gat aac ttc cag ctg
Met His His His His His Thr Ala Ala Ser Asp Asn Phe Gln Leu
tcc cag ggt ggg cag gga ttc gcc att ccg atc ggg cag gcg atg gcg
Ser Gln Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala
```

atc Ile	gcg Ala	ggc Gly 35	cag Gln	atc Ile	cga Arg	tcg Ser	ggt Gly 40	ggg Gly	ggg Gly	tca Ser	ccc Pro	acc Thr 45	gtt Val	cat His	atc Ile	144
ggg Gly	cct Pro 50	acc Thr	gcc Ala	ttc Phe	ctc Leu	ggc Gly 55	ttg Leu	ggt Gly	gtt Val	gtc Val	gac Asp 60	aac Asn	aac Asn	ggc Gly	aac Asn	192
ggc Gly 65	gca Ala	cga Arg	gtc Val	caa Gln	cgc Arg 70	gtg Val	gtc Val	ggg Gly	agc Ser	gct Ala 75	ccg Pro	gcg Ala	gca Ala	agt Ser	ctc Leu 80	240
ggc Gly	atc Ile	tcc Ser	acc Thr	ggc Gly 85	gac Asp	gtg Val	atc Ile	acc Thr	gcg Ala 90	gtc Val	gac Asp	ggc Gly	gct Ala	ccg Pro 95	atc Ile	288
aac Asn	tcg Ser	gcc Ala	acc Thr 100	gcg Ala	atg Met	gcg Ala	gac Asp	gcg Ala 105	ctt Leu	aac Asn	ggg Gly	cat His	cat His 110	ccc Pro	ggt Gly	336
						tgg Trp										384
999 999	aac Asn 130	gtg Val	aca Thr	ttg Leu	gcc Ala	gag Glu 135	gga Gly	ccc Pro	ccg Pro	gcc Ala	gaa Glu 140	ttc Phe	atg Met	gtg Val	gat Asp	432
ttc Phe 145	ggg Gly	gcg Ala	tta Leu	cca Pro	ccg Pro 150	gag Glu	atc Ile	aac Asn	tcc Ser	gcg Ala 155	agg Arg	atg Met	tac Tyr	gcc Ala	ggc Gly 160	480
ccg Pro	ggt Gly	tcg Ser	gcc Ala	tcg Ser 165	ctg Leu	gtg Val	gcc Ala	gcg Ala	gct Ala 170	cag Gln	atg Met	tgg Trp	gac Asp	agc Ser 175	gtg Val	528
gcg Ala	agt Ser	gac Asp	ctg Leu 180	ttt Phe	tcg Ser	gcc Ala	gcg Ala	tcg Ser 185	gcg Ala	ttt Phe	cag Gln	tcg Ser	gtg Val 190	gtc Val	tgg Trp	576
ggt Gly	ctg Leu	acg Thr 195	gtg Val	ggg Gly	tcg Ser	tgg Trp	ata Ile 200	ggt Gly	tcg Ser	tcg Ser	gcg Ala	ggt Gly 205	ctg Leu	atg Met	gtg Val	624
gcg Ala	gcg Ala 210	gcc Ala	tcg Ser	ccg Pro	tat Tyr	gtg Val 215	gcg Ala	tgg Trp	atg Met	agc Ser	gtc Val 220	acc Thr	gcg Ala	999 Gly	cag Gln	672
gcc Ala 225	gag Glu	ctg Leu	acc Thr	gcc Ala	gcc Ala 230	cag Gln	gtc Val	cgg Arg	gtt Val	gct Ala 235	gcg Ala	gcg Ala	gcc Ala	tac Tyr	gag Glu 240	720
acg Thr	gcg Ala	tat Tyr	Gly 999	ctg Leu 245	acg Thr	gtg Val	ccc Pro	ccg Pro	ccg Pro 250	gtg Val	atc Ile	gcc Ala	gag Glu	aac Asn 255	cgt Arg	768
gct Ala	gaa Glu	ctg Leu	atg Met 260	att Ile	ctg Leu	ata Ile	gcg Ala	acc Thr 265	aac Asn	ctc Leu	ttg Leu	ggg Gly	caa Gln 270	aac Asn	acc Thr	816

						•										
						gtt Val										1584
						atc Ile										1632
I						ctg Leu 550										1680
						gtc Val										1728
						acc Thr										1776
	_					gtg Val				_		_				1824
						caa Gln										1872
1						gcg Ala 630										1920
	_	_				ggt Gly			_		_			-	-	1968
-	-	_			_	ggt Gly		_		_	_		_	 		2016
_	-			-		ctc Leu										2064
			-	_		aca Thr	_			_		_		_	-	2112
]		_			_	tcg Ser 710						Asn				2160
						acg Thr				tag 730	g	•				2191

```
<210> 12
<223> Description of Artificial Sequence:Ra12-H9-32A fusion
<211> 729
      polypeptide (Ral2-MTB39-MTB32A(N-ter) fusion polypeptide)
<212> PRT
 Met His His His His His Thr Ala Ala Ser Asp Asn Phe Gln Leu
 Ser Gln Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala
  Ile Ala Gly Gln Ile Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile
  Gly Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn
   Gly Ala Arg Val Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu
   Gly Ile Ser Thr Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile
    Asn Ser Ala Thr Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly
    Asp Val Ile Ser Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr
     Gly Asn Val Thr Leu Ala Glu Gly Pro Pro Ala Glu Phe Met Val Asp
     Phe Gly Ala Leu Pro Pro Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly
      Pro Gly Ser Ala Ser Leu Val Ala Ala Ala Gln Met Trp Asp Ser Val
      Ala Ser Asp Leu Phe Ser Ala Ala Ser Ala Phe Gln Ser Val Val Trp
      Gly Leu Thr Val Gly Ser Trp Ile Gly Ser Ser Ala Gly Leu Met Val
       Ala Ala Ser Pro Tyr Val Ala Trp Met Ser Val Thr Ala Gly Gln
       Ala Glu Leu Thr Ala Ala Gln Val Arg Val Ala Ala Ala Ala Tyr Glu
        Thr Ala Tyr Gly Leu Thr Val Pro Pro Pro Val Ile Ala Glu Asn Arg
        Ala Glu Leu Met Ile Leu Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr
         Pro Ala Ile Ala Val Asn Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln
         Asp Ala Ala Met Phe Gly Tyr Ala Ala Ala Thr Ala Thr
          Ala Thr Leu Leu Pro Phe Glu Glu Ala Pro Glu Met Thr Ser Ala Gly
          Gly Leu Leu Glu Gln Ala Ala Val Glu Glu Ala Ser Asp Thr Ala
           Ala Ala Asn Gln Leu Met Asn Asn Val Pro Gln Ala Leu Gln Gln Leu
           Ala Gln Pro Thr Gln Gly Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu
           Trp Lys Thr Val Ser pro His Arg Ser pro Ile Ser Asn Met Val Ser
            Met Ala Asn Asn His Met Ser Met Thr Asn Ser Gly Val Ser Met Thr
            Asn Thr Leu Ser Ser Met Leu Lys Gly Phe Ala Pro Ala Ala Ala
```

Gln Ala Val Gln Thr Ala Ala Gln Asn Gly Val Arg Ala Met Ser Ser 420 425 Leu Gly Ser Ser Leu Gly Ser Ser Gly Leu Gly Gly Val Ala Ala 440 445 Asn Leu Gly Arg Ala Ala Ser Val Gly Ser Leu Ser Val Pro Gln Ala 455 460 Trp Ala Ala Ala Asn Gln Ala Val Thr Pro Ala Ala Arg Ala Leu Pro 470 475 Leu Thr Ser Leu Thr Ser Ala Ala Glu Arg Gly Pro Gly Gln Met Leu 490 485 Gly Gly Leu Pro Val Gly Gln Met Gly Ala Arg Ala Gly Gly Gly Leu 505 Ser Gly Val Leu Arg Val Pro Pro Arg Pro Tyr Val Met Pro His Ser 520 525 Pro Ala Ala Gly Asp Ile Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe 540 535 Ala Asp Phe Pro Ala Leu Pro Leu Asp Pro Ser Ala Met Val Ala Gln 550 555 Val Gly Pro Gln Val Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn 575 570 565 Ala Val Gly Ala Gly Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val 580 585 590 Leu Thr Asn Asn His Val Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe 605 600 595 Ser Val Gly Ser Gly Gln Thr Tyr Gly Val Asp Val Val Gly Tyr Asp 615 Arg Thr Gln Asp Val Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu 630 635 Pro Ser Ala Ala Ile Gly Gly Gly Val Ala Val Gly Glu Pro Val Val 650 645 Ala Met Gly Asn Ser Gly Gly Gln Gly Gly Thr Pro Arg Ala Val Pro 660 665 670 Gly Arg Val Val Ala Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu 675 680 Thr Gly Ala Glu Glu Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala 695 700 Ile Gln Pro Gly Asp Ser Gly Gly Pro Val Val Asn Gly Leu Gly Gln 710 715 Val Val Gly Met Asn Thr Ala Ala Ser <210> 13 <211> 51 <212> DNA <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide
 primer for PCR amplification of Ral2 C-terminal
 fragment of MTB32A

<400> 13

caattacata tgcatcacca tcaccatcac acggccgcgt ccgataactt c

51

<210> 14

<211> 33

<212> DNA

<213> Artificial Sequence

<220> <223> Description of Artificial Sequence:3' oligonucleotide primer for PCR amplification of Ra12 C-terminal fragment of MTB32A	
<400> 14 ctaatcgaat tcggccgggg gtccctcggc caa	33
<210> 15 <211> 48 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence:5' oligonucleotide primer containing enterokinase recognition site for PCR amplification of DPPD mature secreted form	
<400> 15 caattagaat tegaegaega egaeaaggat eeacetgaee egeateag	48
<210> 16 <211> 33 <212> DNA <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence:3' oligonucleotide primer containing enterokinase recognition site for PCR amplification of DPPD mature secreted form	
<400> 16 caattagaat teteagggag egttgggetg ete	33
<210> 17 <211> 30 <212> PRT <213> Artificial Sequence	
<220> <223> Description of Artificial Sequence:Ral2(short) polypeptide	
<pre><400> 17 Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gln Gly Phe</pre>	
<210> 18 <211> 128 <212> PRT <213> Artificial Sequence	

```
<220>
<223> Description of Artificial Sequence:Ra12(long)
      polypeptide
<400> 18
Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gln Gly Phe
                                     10
Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Lys Leu
                                 25
             20
Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly Leu Gly Val Val
                             40
                                                 45
Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val Val Gly Ser Ala
                        55
                                             60
    50
Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val Ile Thr Ala Val
                     70
                                         75
Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala Asp Ala Leu Asn
                                     90
                 85
Gly His His Pro Gly Asp Val Ile Ser Val Thr Trp Gln Thr Lys Ser
                                105
                                                     110
Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu Gly Pro Pro Ala
        115
                            120
                                                125
<210> 19
<211> 34
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:5'
      oligonucleotide primer, HindIII site, for PCR
      amplification of human mammaglobin
<400> 19
gcgaagctta tgaagttgct gatggtcctc atgc
                                                                   34
<210> 20
<211> 36
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:3'
      oligonucleotide primer, XhoI site, for PCR
      amplification of human mammaglobin
<400> 20
                                                                   36
cggctcgagt taaaataaat cacaaagact gctgtc
<210> 21
<211> 7
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: Met-His tag 6aa
```

W. Market

<400> 21 Met His His His His His His 1 5

<210> 22 <211> 4 <212> PRT <213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:enterokinase recognition site

<400> 22 Asp Asp Asp Lys